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POPULATION ECOLOGY - ORIGINAL RESEARCH

Are host–parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels

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Abstract Natural populations often face multiple mortality sources. Adaptive responses to one mortality source might also be beneficial with respect to other sources of mortality, resulting in "reinforcing adaptations"; or they might be detrimental with respect to other sources of mortality, resulting in "conflicting adaptations". We explored these possibilities by testing experimentally if the responses of guppies (*Poecilia reticulata*) to the monogenean ectoparasitic worm *Gyrodactylus* differed between populations adapted to different predation regimes. In experimental stream channels designed to replicate the natural environment, we exposed eight guppy populations (high-predation and low-predation populations from each of four separate rivers) either to their local *Gyrodactylus* parasites (infection treatment) or to the absence of those parasites (control). We found that infection dynamics varied dramatically among populations in a repeatable fashion, but that this variation was not related to the predation regime of origin. Consistent with previous work, high-predation guppy females gained more mass, had lower reproductive investment, and had more but smaller embryos than did low-predation females. Relative to control (no parasite) channels, guppies from treatment (infected) channels gained less mass but produced similar numbers and sizes of embryos—and thus had a higher reproductive effort. However, no interaction was evident between infection treatment and predation regime. We conclude that parasitism by *Gyrodactylus* and predation are both likely selective forces for guppies, but that adaptation to predation does not have an obvious deterministic effect on host–parasite dynamics or on life-history traits of female guppies.

Keywords *Poecilia reticulata* · Life-histories · Infection dynamics · Predation · Parasitism

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Introduction

Natural populations experience a wide variety of extrinsic sources of mortality that can have important consequences for the fitness of individuals and the dynamics of populations: e.g., predation (Stoks and McPeek 2003), parasitism (Mouritsen and Poulin 2010), resource limitation (Holland and DeAngelis 2009), and pollution (Billoir et al. 2007). Populations tend to adapt to these local mortality sources by evolving traits that reduce mortality and increase the chances of reproduction (Stearns 1977, 1992; Roff 1992).

Most studies of these responses tend to focus on overall mortality rates or on mortality from one specific cause, such as predation. The natural situation, however, is much more complex because populations typically face multiple mortality sources that might simultaneously include a variety of different predators, parasites, competitors, and abiotic stressors. Under such conditions, predicting evolutionary responses is difficult because one needs to account not only for multiple sources of mortality but also for the ways in which they interact.

Interactions between different sources of mortality could be reinforcing or conflicting with respect to adaptation. As an example of reinforcing effects, life-history theory predicts that elevated mortality at a given stage will generally select for particular adaptations of age at maturity, reproductive allocation and body growth, irrespective of the specific source of that mortality (Roff 1992; Stearns 1992). As an example of conflicting effects, induced morphological changes in *Daphnia* can decrease mortality due to fish predation, while increasing the risk of parasitism (Yin et al. 2011). Whether or not adaptations to different mortality sources have reinforcing or conflicting effects has important evolutionary implications. In particular, it would seem easier for a population to adapt to reinforcing selective pressures, because the same adaptive trait changes will decrease mortality from multiple sources. In contrast, conflicting influences dictate that trait changes that are adaptive with respect to one mortality factor could well be maladaptive with respect to other factors.

We are specifically interested in the interaction between two common sources of mortality: predation and parasitism. On the one hand, reinforcing effects could be possible because both predators and parasites can cause high mortality, which should generally favor the evolution of earlier age at maturity, higher reproductive investment, and more but smaller offspring (Law 1979; Roff 1992; Stearns 1992; Reznick et al. 1996). Thus, adaptation to parasitism could aid adaptation to predation. On the other hand, conflicting effects are also possible because predation can select for behaviors, such as increased grouping (Seghers 1974; Seghers and Magurran 1995), that protect against predation but might increase exposure to directly transmitted parasites (Hatcher et al. 2006). Also, some aspects of adaptation to parasites, such as the immune response (Dybdahl and Storfer 2003), may have little influence on adaptation to predators. We have commenced a research effort in Trinidadian guppy (*Poecilia reticulata*) populations to consider these various possibilities.

Guppies, predators, and parasites

The Trinidadian guppy is a sexually dimorphic live-bearing freshwater fish endemic to north-eastern South America. This species is frequently used in evolutionary studies due to its dramatic and rapid adaptation to its local environment (reviews: Endler 1995; Houde 1997; Magurran 2005). The best-studied environmental contrast is predation intensity, commonly classified as "high" (many dangerous predatory fishes that have major effects on guppy demography) versus "low" (fewer and less dangerous predatory fishes that have only minor effects on guppy demography). This contrast is clearly an important evolutionary force given the ample evidence that high-predation and low-predation guppy populations exhibit genetically based adaptive divergence in a great diversity of traits (reviews: Endler 1995; Houde 1997; Magurran 2005). Furthermore, these differences evolve rapidly when high-predation guppies are introduced into low-predation environments, or when dangerous predators are introduced into formerly low-predation environments (Endler 1980; Reznick and Bryga 1987; Reznick et al. 1996; Gordon et al. 2009).

Despite this focus on the effects of predation regime, parasites could also be very important (van Oosterhout et al. 2006, 2007; Cable and van Oosterhout 2007a; Fraser and Neff 2010; Fraser et al. 2010). Most of the parasite work conducted thus far has focused on *Gyrodactylus*, a genus of ubiquitous host-specific viviparous monogenean ectoparasites of freshwater and marine fishes (Kearn 1994; Harris et al. 2004). Natural guppy populations in Trinidad are infected by up to two species of *Gyrodactylus*: *G. bullatarudis* and *G. turnbulli* (Harris and Lyles 1992; Martin and Johnsen 2007). Both species can be found in sympatry on the same individual guppy, with *G. bullatarudis* commonly found on the anterior end of the fish, particularly on the head and gills, and *G. turnbulli* commonly found on the posterior end, particularly on the caudal fin, peduncle, and anal fin (Harris and Lyles 1992).

After infecting a guppy, each individual *G. turnbulli* gives birth to up to three individuals that are released onto the skin of the fish (Scott 1982). The first daughter is a clone of the mother that is born approximately 24 h after the mother matures, and it carries its own developing embryo inside. The second and third daughters start developing immediately after this first birth, and are themselves born 2.5 and 4.5 days later (Scott 1982). This life cycle characteristic of gyrodactylids allows populations to grow rapidly on an individual host. Transmission to other hosts occurs primarily when the parasites on one host "jump" to another nearby host. This direct transmission, together with rapid population growth on individual fish, leads to epidemic spread of infection through fish populations (Scott and Anderson 1984). Moreover, infections by *Gyrodactylus* can cause high guppy mortality in the laboratory (Scott and Anderson 1984; Cable and van Oosterhout 2007a, b) and in nature (van Oosterhout et al. 2007).

Gyrodactylus is thus a good candidate for the study of whether adaptation to predation influences adaptation to

parasitism. Some previous work has considered this possibility. In particular, wild-caught guppies from a high-predation site on the Aripo River in Trinidad had lower parasite loads and shorter infection durations (when tested in the laboratory) than did guppies from a low-predation site in the same river (van Oosterhout et al. 2003; Cable and van Oosterhout 2007a, b). However, the generality of this apparent high-predation versus low-predation effect is uncertain given that no other populations have been tested in a similar manner, and that guppy traits differ dramatically among populations within a given predation regime (Endler 1978; Reznick et al. 1996; Millar and Hendry 2011).

Our experiment

Our goal was to explore how wild-caught guppies from high- versus low-predation environments differ in (1) the infection dynamics of their sympatric *Gyrodactylus* parasite, and (2) in their life-history responses to *Gyrodactylus* infection. We therefore collected young guppies from high-predation and low-predation populations in four different rivers and placed them into experimental stream channels with and without their local *Gyrodactylus* parasites. Local parasites were used (i.e., each guppy population with its own parasite strain), as opposed to cross-infections, because we were interested in host-parasite interactions that actually occur in nature.

Materials and methods

Experimental design

We conducted two experiments using guppies collected from four different rivers in northern Trinidad: Quare, Aripo, Marianne, and El Cedro (Online Resource 1). In each case, a guppy population was divided into a parasite treatment (with *Gyrodactylus*) and a parasite-free control, each in a different experimental stream channel. Although here the real experimental manipulation relative to the natural population involves the removal of *Gyrodactylus*, our goal is to infer whether the effect of the presence of *Gyrodactylus* differs among populations. We thus follow this logic and refer to the *with parasite* condition as our treatment and the *without parasite* condition as our control. Experiment 1 involved fish collected only from high predation sites in the four rivers, resulting in two infection treatments crossed with four populations. Experiment 2 involved fish from the same high-predation sites, as in experiment 1, paired with low-predation sites from the same four rivers, resulting in two infection treatments crossed with four rivers and two predation regimes in each river. Experiment 1 served as an initial trial testing the feasibility and effect size of our infection treatment in the experimental stream channels. Also, because the experimental procedures were identical for the two experiments, data from experiment 1 allowed us to consider whether results were repeatable for the four populations that were included in both experiments. Experiment 2 then served as our formal test for effects of predation and parasitism.

The experimental stream channels were the same as those used in previous work on guppies (Palkovacs et al. 2009; Bassar et al. 2010): we used eight channels for experiment 1 and 16 for experiment 2. These channels are 0.5 m wide by 3 m long by 0.2 m deep, and throughout the experiments receive flowing water from a tributary to the Arima River. Seven days prior to the introduction of fish, the channels were thoroughly cleaned and gravel was added, making them available for natural colonization by the invertebrates and algae that provide food for the guppies. Predators were not included because we were not interested in the direct effects of predation risk on *Gyrodactylus*–guppy interactions, but rather the ecological implications of evolving under different predation environments.

It is important to note that our use of water and invertebrates/algae from the Arima River provides a useful standardization in the sense that none of the other populations experienced these specific conditions in nature—because they all came from other rivers. It is certainly possible that the experimental results might have differed in another "common garden" (water from another river), but it is at least known that the specific channels and conditions we used have been effective in revealing differences between guppy populations from other rivers (Palkovacs et al. 2009; Bassar et al. 2010). We are therefore confident that our results reveal host–parasite-mediated differences between populations.

Guppy populations and collections

During September (experiment 1) and November (experiment 2) of 2009, we collected guppies from four rivers: Quare, Aripo, Marianne, and El Cedro (Online Resource 1). Guppies in the first three rivers represent different guppy lineages, likely separated for millions of years (Fajen and Breden 1992; Willing et al. 2010). Guppies in the El Cedro river represent the same lineage as those in the Aripo river, but are nevertheless geographically separate and genetically differentiated (Willing et al. 2010). Within the El Cedro, the high-predation guppies are natural (as they are in the three other rivers), whereas the low-predation guppies are descendants of the downstream high-predation guppies that were introduced upstream of a waterfall in 1981 into a previously guppy-free environment (Reznick and Bryga 1987). Predatory fauna across the south-slope rivers (El Cedro, Aripo and Quare) consists primarily of

cichlids (*Crenicichla alta* and *Aequidens pulcher*), whereas the Marianne site is dominated by eleotrids (*Eleotris pisonis* and *Gobiomorus dormitor*: Reznick and Bryga 1996).

The specific high-predation and low-predation collection sites within each river were chosen based on previous studies establishing the local predation regime by (1) the presence/absence of dangerous predators (Endler 1978; Reznick et al. 1996; Rodd and Reznick 1997; Magurran and Phillip 2001), and (2) direct measurements of mortality rate (Reznick et al. 1996; Bryant and Reznick 2004; Weese et al. 2010). As far as is known from previous studies, the basic predation regime (high or low) is consistent through time at each of these sites, although predation intensity might well vary. Thus, our inferences relate to the basic predation rather than the specific predation intensity.

At each collection site (four for experiment 1, eight for experiment 2), 30 mature males and 30 juvenile females were collected and transported in individual c.240-g (8-oz) Whirl–Pak bags (Fisher Canada) to our laboratory in Trinidad. Juvenile females were selected (based on their small size and poorly developed "gravid spot") to maximize the chance that they were virgins. On arrival at the laboratory, the fish were anaesthetized in 0.02% Tricaine Methanesulfonate (Finquel MS222; Fisher Canada) (1:8,000) buffered to a neutral pH using $NaHCO_3$. The parasite load of each individual was then determined by scanning its entire surface using a dissecting microscope under illumination by a cold light source. Each fish was also measured (standard length to the nearest mm), weighed (mass to the nearest mg), and given an individual intra-dermal mark with non-toxic Visible Implant Elastomer dye (Northwest Marine Technology). This marking methodology has been used in numerous guppy studies and has no apparent influence on guppy survival (Reznick et al. 1996; Bryant and Reznick 2004; Gordon et al. 2009; Weese et al. 2010).

After the above processing, *Gyrodactylus* infections were eliminated from the fish by treating them with an aqueous solution of *N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine (cyromazine), (Lice And Anchor Worm Treatment; Ecological Laboratories), according to the manufacturer's recommendations. During and after the parasite removal period, the guppies were held in gender- and population-specific recovery tanks, where they were scanned for parasites (as above) over five consecutive days. Individuals found to still carry parasites on any of these days were immediately transferred to individual 1-L aquaria, and again treated as above. By the end of the 5-day period, no parasites were observed on any of the fish. All fish were then kept in recovery aquaria for a period of 4 weeks, which allowed them to recover full susceptibility to future *Gyrodactylus* infections (Scott 1985). During this period, no mature males and no fry were found in the female tanks—confirming that they were indeed virgins.

Experimental infection

After the 4-week recovery period, all fish were measured and weighed in the same manner as described above. Fish from each population were then randomly divided into two experimental groups: one to be infected and one to serve as an uninfected control. Ten males and ten females were then introduced into each of the experimental channels. The resulting densities were below those normally seen in the wild (Rodd and Reznick 1997) in the hope that life-history traits would not be influenced by variation in density that resulted from any parasite-induced mortality during the experiment.

To initiate an epidemic outbreak in the infection treatment channels, a few additional male guppies were collected from each of the field sites. One naturally infected male with two to four *Gyrodactylus* attached to his caudal peduncle or caudal fin was measured and weighed as above, and then introduced to the infection treatment channels 2 days after introduction of the other fish. Live *Gyrodactylus* on experimental guppies cannot be identified to species level, but the above location of attachment on the fish is consistent with *G. turnbulli* (Harris and Lyles 1992). The same procedure was implemented by introducing an uninfected male (confirmed by scanning three times) into the no-infection control channels. The day of introduction of the infected or uninfected male was considered to be experimental day 0, after which fish survival and infection dynamics were tracked for 4 weeks by counting the individual parasite loads on each fish on every second day (using the above methodology). This process was replicated for the control channels to ensure that guppies in the control treatments were indeed uninfected and that they experienced similar handling conditions to guppies in the infected treatments.

Two small modifications were required to the above procedure for experiment 2. In the El Cedro low-predation infection treatment, the introduced infected male shed its parasites on the first day. We therefore re-infected this male using two parasites from another recently caught male, and re-introduced him into the channel. Similarly, in the Aripo high-predation infection treatment, the introduced infected male shed its parasites twice and so was re-infected twice. In both cases, the day of the final re-introduction of the infected male was considered as day 0.

After 4 weeks, all fish were captured from the channels, measured (standard length to the nearest 0.1 mm), weighed (to the nearest 0.1 mg), and euthanized with an overdose of MS222. The embryos in each female were then counted and their stage of development determined according to Haynes (1995) identification keys. In addition, the total fresh mass of all embryos was measured for each female. The remaining guppies that were not used in the experiment

Table 1 Characterization of *Gyrodactylus* infection dynamics of Trinidadian guppies (*Poecilia reticulata*) in the eight experimental stream channels from experiment 2

River	Predation regime of wild population	Prevalence in the wild (%)	Max. prevalence during experiment (%)	Time to peak of infection (days)	Time to exclusion (days)	Max. duration of infection (days)	Max. number of parasites	Max. parasite load/individual	Mortality (%)
Quare	Low	17.5	88.9	27	–	20	305	151	28.5
	High	35.8	19	11	11	12	17	13	33.3
El Cedro	Low	7.1	4.74	1	5	6	2	2	33.3
	High	24.2	73.68	27	–	18	72	17	19.0
Marianne	Low	7.5	70	27	–	18	40	6	23.8
	High	28.5	95	25	–	27	111	21	4.7
Aripo	Low	38.2	52.38	19	26	18	22	10	14.2
	High	43.2	9.52	5	11	11	5	4	19.0

were euthanized with an overdose of MS-222. All the procedures in the experiments were in accordance with ethical norms and approved by the McGill University Animal Use Committee in the protocol No. 5759.

Data evaluation and statistical analysis

Data were categorized at individual- and population-level parameters. The former relate the phenotypic response of each individual fish, and the latter to each individual experimental stream channel. Parasite-related parameters recorded at the population level included maximum prevalence of infection (highest percentage of the population infected on a given day), peak of infection (day with the highest total parasite abundance), time to peak of infection, time to exclusion (time until all parasites in the fish population had disappeared), maximum duration of infection (longest individual infection), maximum number of parasites (highest total parasite abundance) and maximum parasite load per individual (highest parasite load on an individual fish at any given day). In three populations (Table 1), the parasite population was still growing at day 27. We acknowledge the possibility that parasite levels might have reached much higher levels; however, for the purpose of our analysis, differences observed during the length of the experiment represent plausible differences in host-parasite dynamics between populations.

The individual phenotypic responses (growth, reproductive effort, number of embryos, embryo mass, and embryo development) of female guppies to *Gyrodactylus* infection were analyzed using general linear models in R version 2.12.2011-02-22 (R Core Development Team 2008). In these models, predation regime of origin (high or low: for the second experiment only), experimental treatment (infected or control), and their interaction were treated as fixed factors, whereas river of origin was considered as a random factor, mainly because our conclusions about river effects are not intended to be specific to those rivers, but to the overall difference between high- and low-predation environments. The individual-based covariates included in the analysis were initial body mass at introduction into the experimental channels and the stage of embryo development. Parasite load-related measurements were entered as covariates to test the regression relationship between parasite load and the strength of female guppy response. However, these proved non-significant and were therefore dropped from all analyses. In the particular case of female growth, neither initial mass nor embryo development was statistically significant, so these covariates were dropped from the model. Response variables, each considered in a separate analysis, were (1) female growth over the 27 days of the experimental infection (males were excluded from this analysis because they stop growing at sexual maturity: Reznick 1982), (2) female reproductive effort (proportion of female growth due to embryonic fresh mass), (3) number of embryos per female, and (4) total mass of embryos per female. In addition to the individual phenotypic response to infection, duration of infection was calculated for each fish as the number of consecutive days it was observed infected during the entire length of the experiment. A few fish became infected, then eliminated their parasites, and then became re-infected. For these individuals, the duration of infection was calculated as the total number of days when parasites were observed for at least two consecutive sampling periods. Fish that were infected on only one sampling date were assigned an infection duration of 1 day. Mean parasite load was calculated for each fish as the mean number of parasites observed during the length of its infection.

Normality was evaluated using Shapiro–Wilk tests and homoscedasticity was examined using Bartlett's tests. Only for the duration of infection and mean parasite load was the assumption of normality violated ($W = 0.968$, $P = 0.012$; $W = 0.505$, $P \leq 0.0002$, respectively). Transformations did not improve normality. These variables were then analyzed

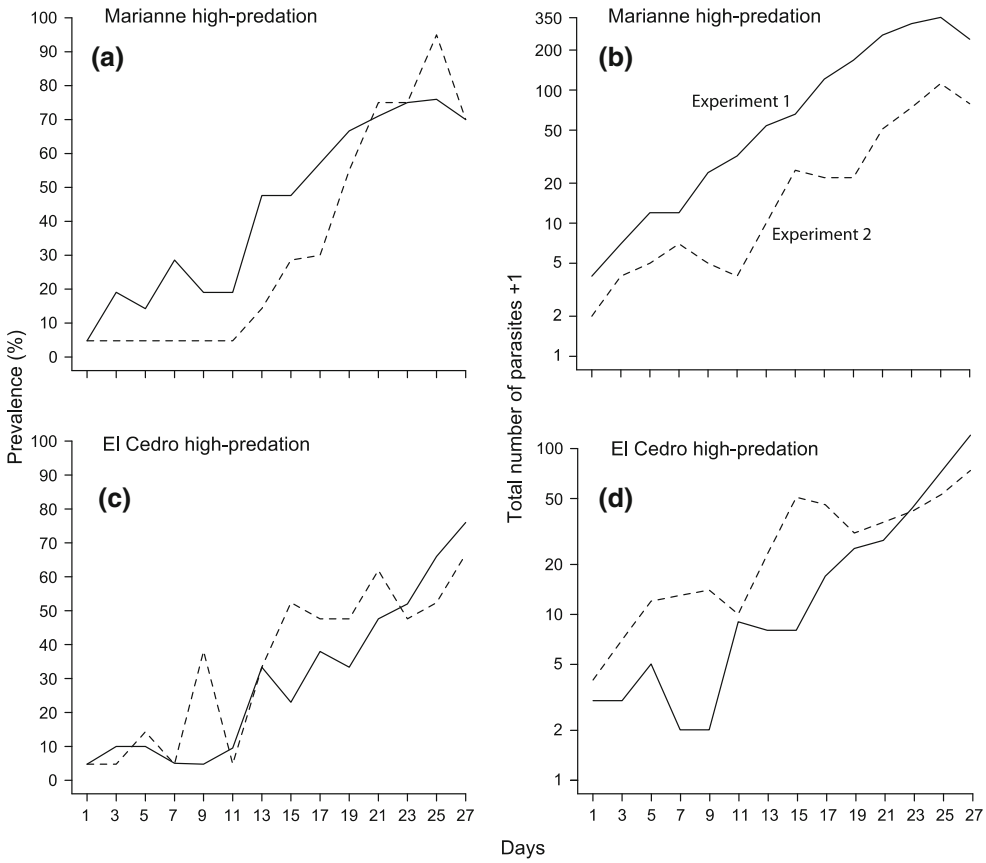

Fig. 1 Epidemic dynamics in Marianne (**a**, **b**) and El Cedro (**c**, **d**) high-predation Trinidadian guppies (*Poecilia reticulata*) in experiment 1 (*solid line*) and experiment 2 (*dashed line*). **a**, **c** Prevalence of infection (percentage of the population infected on a given day) and **b**, **d** *Gyrodactylus* population dynamics. Data in (**b**) and (**d**) have been log-transformed

using Kruskal–Wallis nonparametric tests with predation as a main effect and rivers pooled together per predation regime. Males and females were pooled together for each river.

Several additional details are pertinent. First, fish that died during the experiment were excluded from analyses of phenotypic responses. Second, the infection did not establish or spread in several of the experimentally infected groups in the second experiment (Table 1). These populations, including their respective controls, were therefore removed from the analysis of guppy phenotypic responses. This resulted in an unbalanced design with three low-predation populations (Quare, Aripo, and Marianne) and two high-predation populations (El Cedro and Marianne). The level of significance for all analyses was set at $P < 0.05$.

Results

Infection dynamics

Infection levels recorded on the fish immediately after their collection from the wild varied dramatically among rivers, both between and within predation regimes (Table 1). Within each river, however, high-predation sites had a higher *Gyrodactylus* prevalence than those from low-predation sites ($t = 3.851$; $df = 3$; $P = 0.030$).

In the experimental stream channels, the introduction of a *Gyrodactylus*-infected male led to remarkably similar infection dynamics for the four (high-predation) guppy populations that were replicated between experiments 1 and 2. In both cases, parasites spread and established infections in the Marianne and El Cedro guppy populations (Fig. 1a, c) but did not spread to new hosts and were soon eliminated in the Quare and Aripo guppy populations (data not shown). Also in both experiments, the total *Gyrodactylus* population peaked at higher levels in Marianne guppies than in El Cedro guppies: 351 versus 120, respectively, in experiment 1 and 111 versus 72, respectively, in experiment 2 (Fig. 1b, d). These comparisons indicate that infection dynamics are a repeatable property of host–parasite interactions for a given population, thus validating our use of only a single population per river (experiment 2) for comparing high-predation and low-predation guppy-*Gyrodactylus* interactions.

In experiment 2, infection dynamics differed among populations but did not show a consistent association with the predation regime of origin (Fig. 2)—as two comparisons

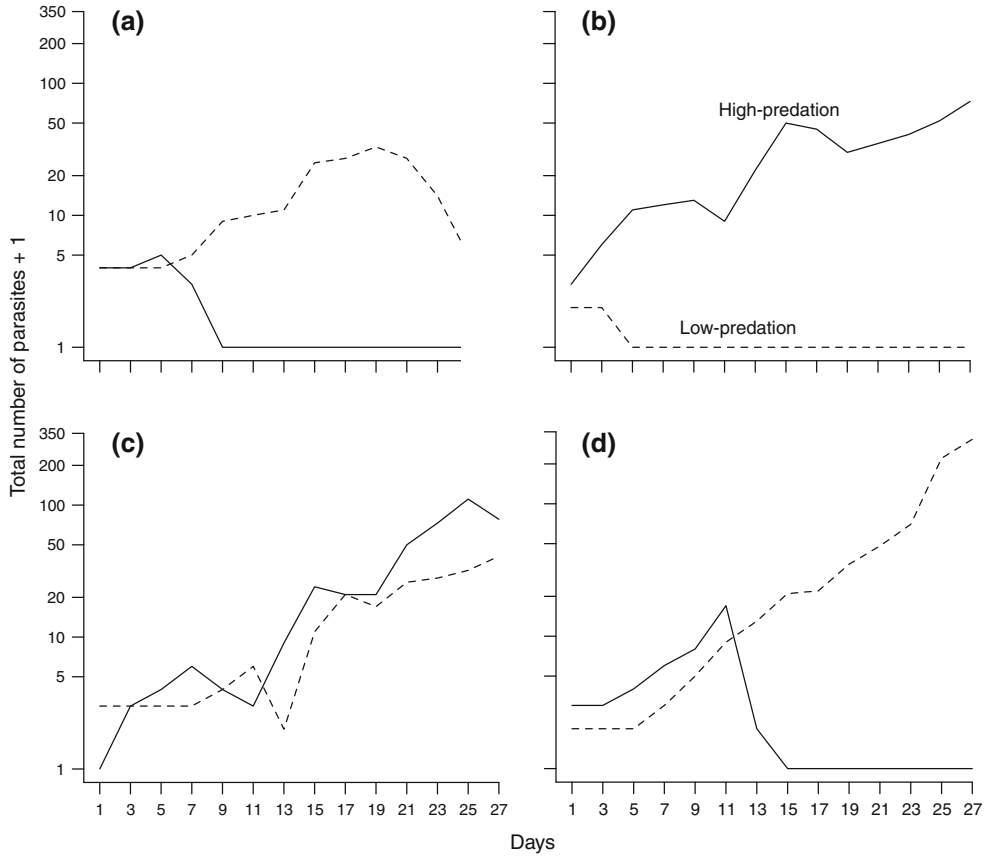

Fig. 2 *Gyrodactylus* population dynamics on high-predation guppies (*solid lines*) and low-predation guppies (*dashed lines*) from the four rivers used in experiment 2: **a** Aripo, **b** El Cedro, **c** Marianne and **d** Quare. Data in all panels have been log-transformed

reveal. First, the infection did not spread in both a low-predation population (El Cedro) and two high-predation populations (Aripo and Quare). Second, for populations where the infection did spread, predation regime did not significantly affect mean parasite load (Kruskal–Wallis test: $H = 1.413$; $df = 1$; $P = 0.234$) or the mean duration of infection (Kruskal–Wallis test: $H = 0.529$; $df = 1$; $P = 0.466$).

Guppy responses

In experiment 2, guppy mortality was similar between the infected and control channels (Table 1): average mortality in infected channels was 22.0% compared to 22.6% for control channels. Among the infected channels, El Cedro and Quare low-predation channels had the highest mortality (33 and 28%, respectively). Among the control channels, highest mortality was observed in El Cedro and Marianne low-predation, 48 and 28%, respectively.

Female growth was reduced in the infection treatment channels compared to the control channels, and it was higher in guppies from high-predation populations than from low-predation populations (Fig. 3a; Table 2). The interaction between predation and infection did not have a significant effect (Table 2).

When controlling for initial mass, females from high-predation environments had more embryos, greater total embryo mass, and higher reproductive effort than did those from low-predation environments (Fig. 3c, d; Table 2). Parasite infection (i.e., infected versus control channels) had no detectable influence on the number of embryos or total embryo mass (Table 2), but it did influence our measure of reproductive effort. In particular, females from infection treatment channels had an approximately 4% greater reproductive effort than did females from the uninfected channels (Fig. 3d; Table 2). This result occurred because females in the infection treatment channels attained a similar embryo mass despite their reduced growth. However, no interaction was evident between predation regime and infection treatment for any of the variables. Interestingly, females in low-predation channels exposed to parasites had a reproductive effort that was close to that of high-predation females not exposed to parasites (Fig. 3d).

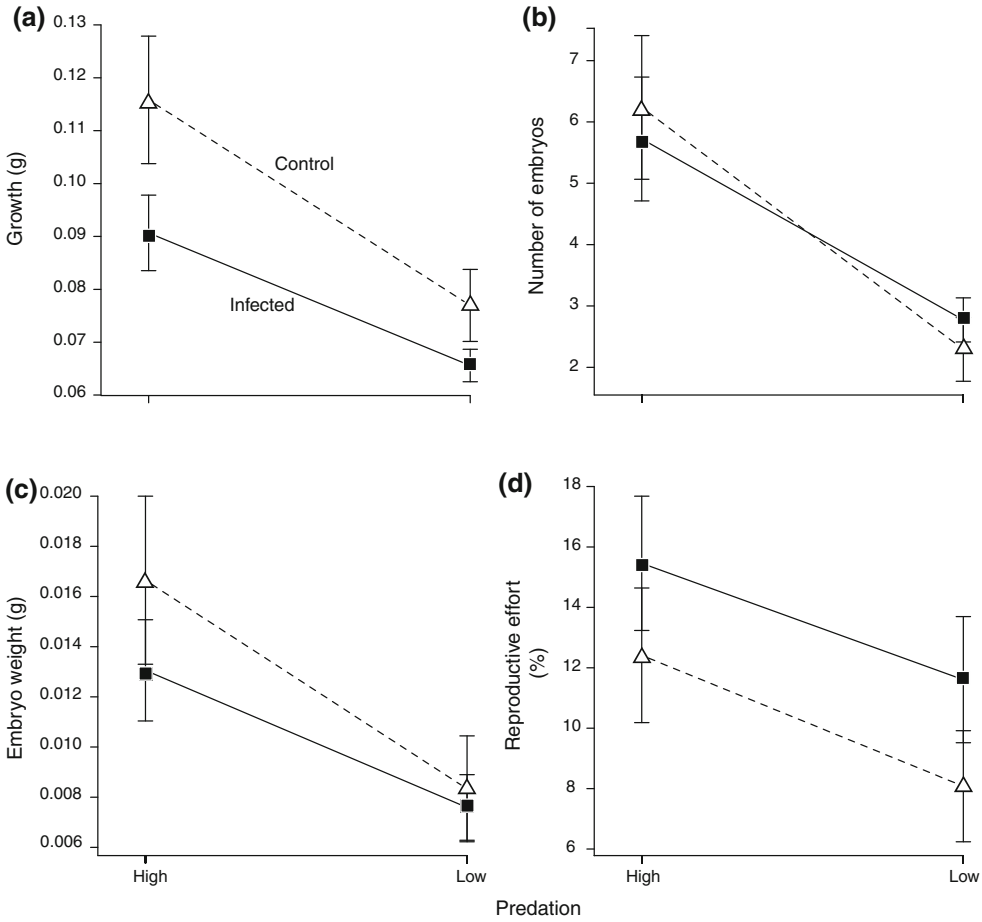

Fig. 3 Differences in phenotypic traits between females from infected and control channels and high-predation and low-predation experimental populations from experiment 2: **a** growth, **b** number of embryos, **c** embryo mass, and **d** reproductive effort (proportion of female growth due to embryonic fresh mass). Only those populations in which infections did establish were analyzed, along with their respective controls. *Dashed line–open triangle*, control treatment; *solid line–closed square*, infected treatment. *Error bars* ±1SE

Table 2 Statistical analyses of female life-history traits in experiment 2

Variable	Female growth		Number of embryos		Embryo mass		Reproductive effort	
	F (*df*)	*P*	*F* (*df*)	*P*	*F* (*df*)	*P*	*F* (*df*)	*P*
Fixed factors								
Infection	5.118 (1,74)	**0.026**	0.076 (1,72)	0.810	1.465 (1,72)	0.230	6.020 (1,72)	**0.016**
Predation	20.546 (1,74)	**<0.001**	29.683 (1,72)	**<0.001**	22.799 (1,72)	**<0.001**	8.240 (1,72)	**0.005**
Predation × infection	0.865 (1,74)	0.355	0.126 (1,72)	0.753	0.0108 (1,72)	0.917	0.317 (1,72)	0.575
Initial mass	–	–	15.056 (1,72)	**0.009**	60.762 (1,72)	**<0.001**	58.864 (1,72)	**<0.001**
Embryo development	–	–	22.821 (1,72)	**<0.001**	47.501 (1,72)	**<0.001**	32.744 (1,72)	**<0.001**
	Standard deviation		Standard deviation		Standard deviation		Standard deviation	
	Intercept	Residual	Intercept	Residual	Intercept	Residual	Intercept	Residual
Random factors								
River	0.011	0.032	0.000	2.819	0.000	0.006	0.000	6.354

All traits were analyzed using general linear models with "infection" and "predation" as fixed effects. "River" was entered as a blocking factor. "Initial mass" and "Embryo development" were removed from female growth analysis due to non-significance

Significant values ($P < 0.05$) shown in bold

Discussion

We explored *Gyrodactylus* infection dynamics and guppy traits for four replicate instances (i.e., different rivers) of guppy population divergence between high-predation and low-predation environments. In a first experiment, we found that infection dynamics in experimental stream channels differed in a repeatable way among guppy populations, but a second experiment showed that this variation was not consistently associated with predation regime. We also

found that guppy traits differed consistently between high-predation and low-predation guppies and were influenced by whether or not parasites were present in the channels, but the effects of predation regime of origin and parasite presence did not interact. In the following sections, we first discuss in more detail the nature of infection dynamics and then further consider effects on guppy traits. We close with a discussion of how our data motivate the logical next experimental steps in examining the interactions between predation and parasitism in this system.

Impact of predation regime on infection dynamics

The lack of a consistent association between predation regime of origin for the guppies and infection dynamics of their local parasites was evident in several comparisons. First, in both experiments, high-predation guppies from the Quare and Aripo rivers were hard to infect with *Gyrodactylus* whereas high-predation guppies from the Marianne and El Cedro rivers were not. Second, of the four populations in which infections persisted for the full duration of experiment 2, two were high-predation and two were low-predation. Finally, none of our measures of dynamics differed between high and low predation sites, despite higher prevalence of infection in wild fish from high-predation sites at the time of collection from the field. This is in contrast to the results of previous studies using only the Aripo river, where experimentally infected individual guppies from a high-predation site (Lower Aripo) had lower parasite loads and shorter duration of infection than those from a low-predation site (Upper Aripo) (van Oosterhout et al. 2003). It is feasible that the observed differences between our results and those previously reported are due to particularities of the rivers or experimental designs. For instance, this is the first time that *Gyrodactylus* dynamics and guppy response of eight different populations from four rivers have been compared. Moreover, here we report *Gyrodactylus*–guppy dynamics in a setting that closely mimics wild populations in a natural environment, allowing free parasite transmission or parasite avoidance, rather than individual infection trials in a laboratory setting. In addition, it is possible that, in populations where the cost of parasitism is high, guppies have evolved a strong innate immune response that prevents, or at least severely limits, the establishment and spread of the parasite. Such could be the case for this particular river. In a later study, Cable and van Oosterhout (2007b) compared parasite dynamics of a highly virulent *Gyrodactylus* strain in fish from high- and low-predation populations from the Aripo River. They found that parasites had a much lower initial establishment rate on fish from the high-predation population. This fits with our results. By contrast, when they re-infected the fish 53 days later, low-predation Aripo guppies had lower parasite loads and were infected for fewer days than were guppies from the high predation population. It would therefore be valuable to repeat our experiment to examine other aspects of the immune response.

The fact that we did not detect a consistent effect of predation regime of origin on infection dynamics could reflect limitations due to lack of replication. However, infection dynamics showed consistency between the four high-predation populations used in experiments 1 and 2. This consistency increases our confidence in concluding that predation regime was not a crucial driver of infection dynamics, at least in our experimental stream channels. At the same time, the variation among populations suggests that other properties of guppies, their parasites, or the local environments where they co-evolved do indeed influence infection dynamics. Examining these factors will require further experimentation.

Guppy traits

A first important point is that our results closely parallel the previously documented differences in growth and life history traits between high-predation and low-predation guppies. Most obviously, guppies from high-predation sites have more embryos, greater total embryo mass, and higher reproductive effort (Reznick and Endler 1982; Reznick and Bryga 1987, 1996; Reznick 1989; Gordon et al. 2009; Fig. 3). A second important point is that, regardless of predation regime, female guppies in infected channels grew more slowly and had higher reproductive effort (proportion of growth composed of embryonic fresh weight) than did female guppies in the control channels. A third important point is that no interaction was evident between predation regime and parasite treatment: that is, divergence owing to evolutionary history of predation regime did not influence responses to local *Gyrodactylus* parasites.

The reduction of female growth in the infection treatment channels might be explained either by (1) a negative impact of *Gyrodactylus* infection on host health, or (2) the activation and maintenance of an energetically costly immune response. The first possibility implies a strictly pathological consequence of the infection, whereas the second possibility could represent an adaptive host response. The parasite loads in our experiments are significantly lower than those typically observed in the laboratory (Scott 1982; Scott and Anderson 1984; Scott 1985) or in field populations (Harris and Lyles 1992; van Oosterhout et al. 2003, 2006; but see Faria et al. 2010). Interestingly, Kolluru et al. (2009) reported that infected first-generation male descendants from the Quare and Madamas low-predation populations increased their foraging behavior when experimentally infected with *Gyrodactylus*. If this is true in wild populations, such compensatory foraging behavior will increase carotenoid intake for its allocation to immune

response (Kolluru et al. 2006), and support the hypothesis that the activation and maintenance of an immune response against *Gyrodactylus* has a great energetic cost. Whether or not these responses then influence susceptibility to predation is not known, although the pathological clamping of fins that typically occurs as a consequence of infection is likely to increase susceptibility to predation due to reduced mobility. This warrants further investigation.

Despite having a lower growth rate, females in infection treatment channels did not have lower total embryo mass, which thus means that their reproductive effort increased relative to those in control channels (Fig. 3b, d). This response is consistent with expectations for a host infected with parasites (like *Gyrodactylus*) that have low initial population growth followed by rapid proliferation. The basic idea is that infections that will be potentially debilitating in the future should provoke the transfer of resources from growth to reproduction (Forbes 1993). Interestingly, this particular plastic response is likely to interact with adaptation to high predation, which also favors increased reproductive effort in guppies (Endler and Reznick 1982; Reznick and Bryga 1987; Reznick et al. 1996) and other taxa (Stibor 1992; Gliwicz 2007). Our own data confirm this idea because the relative increase in reproductive investment associated with either high predation or parasite infection was almost identical (Fig. 3), and because *Gyrodactylus* infection levels were higher in wild high-predation than low-predation guppy populations, both in our study and in previous studies (Martin and Johnsen 2007; Fraser and Neff 2010). Thus, our results on guppy life-history traits suggest that responses to parasites and predators are reinforcing rather than conflicting.

The way forward

Our study shows that *Gyrodactylus* infection dynamics differ greatly among guppy populations from different rivers, and that infection of a guppy population influences guppy growth and life history. Although we found no interaction between predation regime of the guppy populations and the dynamics or effects of parasites, our study provides evidence for reinforcing responses to these factors. In essence, guppy response to *Gyrodactylus* was in the same direction as previous studies have reported for predation (Reznick and Endler 1982; Reznick and Bryga 1987, 1996; Reznick 1989; Gordon et al. 2009). However, further experimentation is needed to reveal the exact nature of the relationship between adaptations to predation and parasitism.

First, it would be useful to perform the same experiment under the risk of predation. For instance, predator chemical cues have been known to alter a number of traits and behaviors in guppies (Dzikowski et al. 2004; Gosline and Rodd 2008). Perhaps these alterations would also modify guppy–*Gyrodactylus* interactions. Second, our experimental design was intended to mimic dynamics that might occur in nature—and so we used each guppy population's local parasites. This means, however, that we cannot separate guppy characteristics from parasite characteristics, which are likely to be associated through co-evolution (van Valen 1973; Price 1980; Ebert and Hamilton 1996; Lively 1999; Kawecki and Ebert 2004). Future work could use the same parasite strain across multiple guppy populations, which would better reveal whether the guppies themselves show responses to parasites that are modified by their predation regime of origin. It would also be useful to cross host genotypes (guppy populations) with parasite genotypes (parasite populations) with environments (presence or absence of predator cues) to see if hosts or parasites are better adapted to each other, and if this depends on the environmental context (Nuismer and Gandon 2008; Gandon and Nuismer 2009). Third, we targeted infection with *G. turnbulli* when *G. bullatarudis* might well have different effects (Cable and van Oosterhout 2007a). Fourth, we used wild-caught fish, whose prior experience could have influenced the results. Although many high-predation versus low-predation guppy differences are genetically based (Reznick 1982; Arendt and Reznick 2005; Karim et al. 2007), some plastic effects are also evident (Rodd and Reznick 1997; Bashey 2006; Gosline and Rodd 2008). An important extension could therefore be to use laboratory-reared fish to disentangle genetic effects from plastic influences.

Conclusions

We found that guppies exposed to a natural epidemic cycle of infection with their local *Gyrodactylus* parasites showed decreased body growth rate and increased reproductive effort, independent of predation regime of origin. We therefore do not have clear evidence that parasite population dynamics are consistently different between guppies from high-predation environments versus those from low-predation environments. However, the parasite-induced decrease in growth rate and increase in reproductive allocation would presumably be less disadvantageous, and even might be advantageous, for high-predation populations that are selected by predators in the same direction. These results suggest that, in this system, adaptation to one agent of mortality (parasitism) could reinforce adaptation to another agent of mortality (predation), although we have here considered only plastic effects of parasitism. Whether genetic divergence in response to parasitism parallels genetic divergence with regard to predation remains to be seen.

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